![Aquaxan logo] Aquaxan™

Technical report

Aquaxan™ HD algal meal use in aquaculture diets:
Enhancing nutritional performance and pigmentation

(TR.2102.001)

The benefits of using Aquaxan HD algal meal in aquaculture diets are reviewed:

- *Aquaxan HD algal meal is prepared from the alga Haematococcus pluvialis.*
- *It is an excellent natural source of algal micronutrients, especially astaxanthin, a unique carotenoid pigment used to improve nutritional performance and pigmentation properties of diets for salmon, trout, shrimp, red seabream, and other marine or tropical aquatic species.*
- *The natural astaxanthin stereoisomer found in Aquaxan HD is the same as that found in the natural food of the aquaculture species targeted, and is the same as the dominant astaxanthin stereoisomer found in their flesh, unlike the synthetic form.*
- *Astaxanthin in Aquaxan HD algal meal has a high bioefficacy.*
- *Trials have shown that Aquaxan HD algal meal has excellent pigmentation properties comparable to synthetic astaxanthin, and that feeding Aquaxan HD algal meal resulted in higher weight gain.*
- *Studies indicate that algal astaxanthin has a higher bio-efficacy than synthetic astaxanthin, especially when used in larval and postlarval shrimp feeds, resulting in improved survival.*
- *Astaxanthin has been attributed vitamin-like properties in fish. Its functions include pro-vitamin A activity, pigmentation, photoresponse and communication, antioxidant, reproduction and development, and a role in immune response mechanisms.*
- *Requirements for astaxanthin are reviewed and recommendations on usage of Aquaxan HD algae meal are provided*
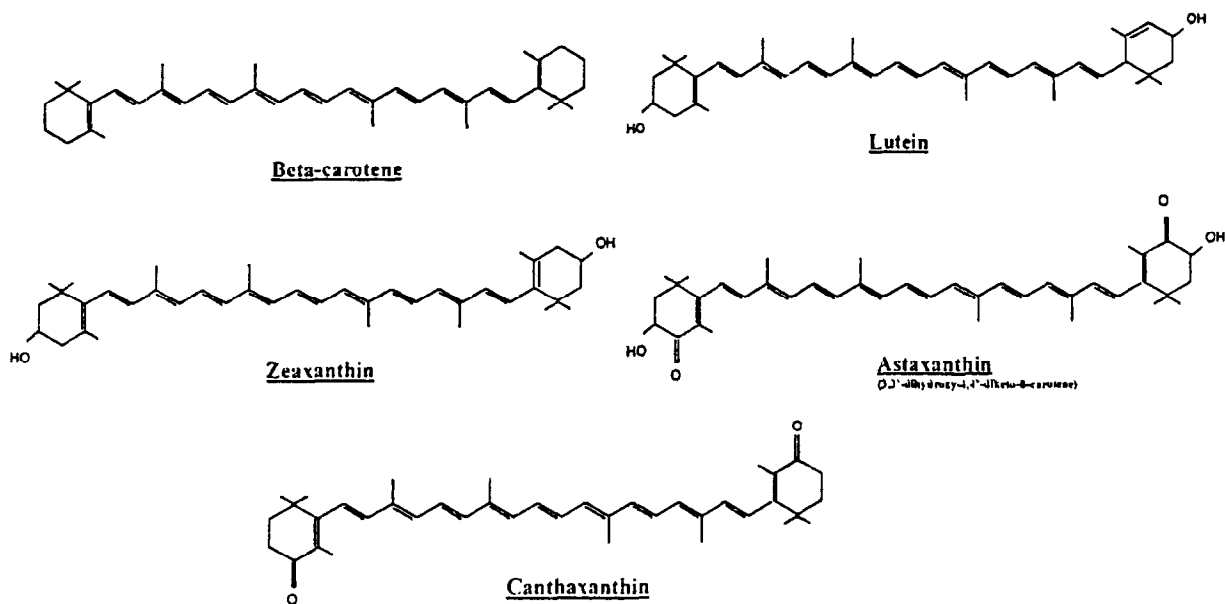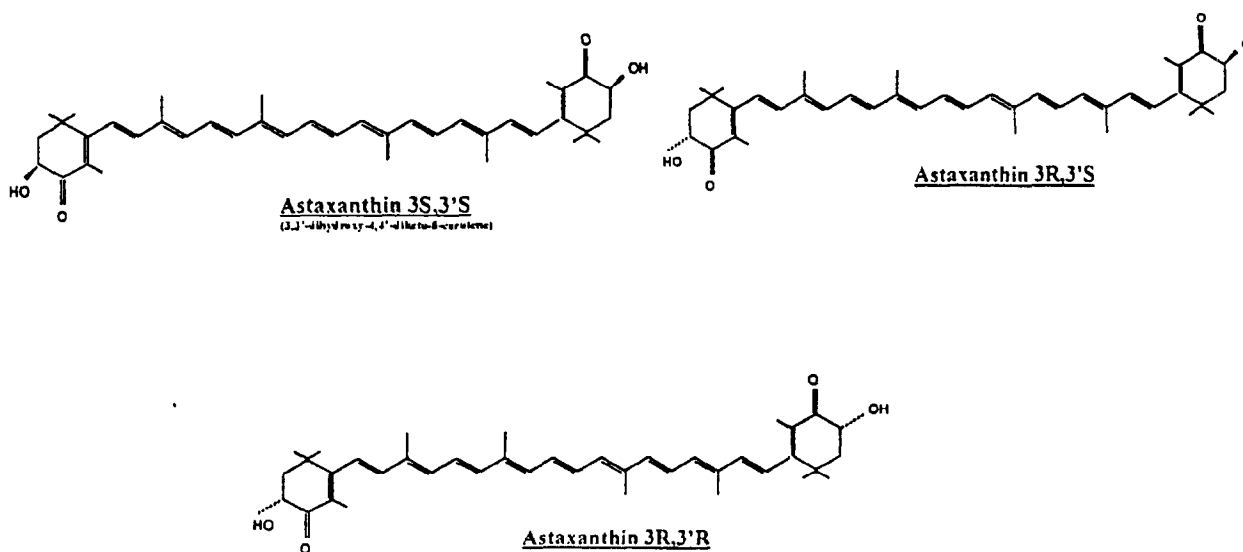
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1. Aquaxan HD algae meal

- Is an algal meal prepared from *Haematococcus pluvialis*. *Haematococcus* spp. are ubiquitous green algae (Chlorophyceae) in the family Volvocales. They are encountered thoughout the world and naturally occur in fresh and brackish waters[1,2,3,4]. When environmental conditions become inhospitable (e. g., drying out of pools), *Haematococcus* cells start reddening, accumulating lipids and **astaxanthin for protection againt photooxidation and other oxidative mechanisms**, while entering a resting phase [1,2,3,4] .

- Is produced by cell-breaking *Haematococcus* algae and gentle drying at low temperature to **ensure minimum degradation and maximum bioavailability of astaxanthin and other micro-nutrients.**

- Is stabilised with the antioxidant ethoxyquin, which ensures a **satisfactory stability when stored at 20°C or below**[6] and comes with a **guaranteed total astaxanthin content**[5].

- Is an excellent source of natural **algal micronutrients, including essential amino acids and polyunsaturated fatty acids** to enhance nutritional performance of aquaculture diets.

- Is particularly rich in **astaxanthin**, a natural **red pigment** that improves **pigmentation of salmon, trout, red seabream and shrimp**, but also has other very important biological functions including **pro-vitamin A activity, communication and photoresponse, protection of lipids against oxidation, protection against light and photooxidation, reproduction, larval development and growth, immune response and health.**

- Contains primarily **esterified astaxanthin, a more stable** form than free astaxanthin in nature[8] although **highly bioavailable**[9].

- Is mainly composed of the **3S,3'S astaxanthin enantiomer**[10], the **same predominant astaxanthin isomer found in wild salmon**[11], while in other sources such as yeast or synthetic astaxanthin, other isomer forms predominate[12].

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Fig. 1. Structure of selected carotenoids

Beta-carotene

Lutein

Zeaxanthin

Astaxanthin
(3,3'-dihydroxy-4,4'-diketo-ß-carotene)

Canthaxanthin

Fig. 2. Astaxanthin enantiomers

Astaxanthin 3S,3'S
(3,3'-dihydroxy-4,4'-diketo-ß-carotene)

Astaxanthin 3R,3'S

Astaxanthin 3R,3'R

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2. Astaxanthin natural occurrence.

- **Astaxanthin is the main carotenoid pigment** found in aquatic animals[13]. It can be found at significant levels in important aquaculture products such as **salmon, trout, red seabream, shrimp, lobster, and fish eggs**[7,14].

- **Astaxanthin cannot be synthesised** by animals and must be provided in the diet as is the case with other carotenoids [7,14]. While salmonids are unable to convert other dietary carotenoids into astaxanthin[7], some species such as crustaceans have a limited capacity to convert closely related dietary carotenoids into astaxanthin, although feeding astaxanthin directly to shrimp rather than precursors results in better pigmentation due to conversion inefficiencies[14,53].

- **Form and level of deposition of astaxanthin differ between tissues**: esterified astaxanthin predominates in the skin, teguments, and eggs, while free astaxanthin is the main form in the flesh, serum and other internal organs of salmon[7]. In shrimp, esterified astaxanthin predominates, except in the ovaries and eggs[17,18]. In red seabream, mostly esterified astaxanthin is found in the skin [17,18]. The more stable esterified form is believed to be an adaptive feature to be able to store astaxanthin in tissues without excessive oxidation[8].

- **Esterified 3S,3'S astaxanthin**, the main astaxanthin enantiomer **in Aquaxan HD algae meal** is the **dominant astaxanthin form in natural foods/preys** of aquaculture species[11]. This 3S,3'S astaxanthin enantiomer is the same as the main enantiomer found in the **flesh of wild salmon**[11]. Salmonids seem to be unable to convert the 3R,3'S enantiomer in synthetic astaxanthin to the natural 3S,3'S form[11]. Fillets from farmed salmon fed synthetic astaxanthin will have characteristically high levels of the 3R,3'S form and can therefore be easily distinguished by analytical means from the wild salmon[11].

Table 1. Main forms of astaxanthin in tissues of important aquaculture species

Tissues	Skin	Flesh	Digestive gland	Ovaries	Serum	Eggs
Species						
Salmonids [7]	Esterified	Free	Free	Free	Free	Esterified
Shrimp [15,16]	Esterified	Esterified	Esterified	Free		Free
Red Seabream[17,18]	Esterified	N.A.	N.A.	N.A.	N.A.	N.A.

N.A.: Not available

Table 2. Form and level of astaxanthin in selected important aquaculture species and potential astaxanthin sources

Form and level of astaxanthin in selected important aquaculture species and potential asta

| | Astaxanthin | | | |
Aquaculture species	Content (mg/kg)	Free/esterified	Main isomer	Reference
Sockeye salmon	26-37	Free,esterified**	$3S,3'S$	11,7
Coho salmon	9-21	Free,esterified**	$3S,3'S$	11,7
Chum salmon	3-8	Free,esterified**	$3S,3'S$	11,7
Chinook salmon	8-9	Free,esterified**	$3S,3'S$	11,7
Pink salmon	4-6	Free,esterified**	$3S,3'S$	11,7
Atlantic salmon	3-11	Free,esterified**	$3S,3'S$	11,7
Rainbow trout	1-3	Free,esterified**	$3S,3'S$	7
salmon eggs	0-14	esterified***	N.A.	19,20
Red seabream	2-14	esterified***	N.A.	17,18
Red seabream eggs	3-8	N.A.	N.A.	20
Peneaus monodon	10-150	Esterified,free**	$3S,3'S$	16
Lobster		Esterified,free**	N.A.*	12, 37
Astaxanthin sources				
Copepods	39-84	esterified***	N.A.*	7
Krill	46-130	esterified***	$3R,3'R$	7
Krill oil	727	esterified***	$3R,3'R$	7
Crayfish meal	137	esterified***	N.A.*	7
Arctic shrimp	1160	esterified***	$3S,3'S$	7
Yeast (*Pfaffia rhodozyma*)	30-800	esterified***	$3R,3'R$	7
Synthetic astaxanthin	80,000	free	$3R,3'S$	7
Haematococcus pluvialis	10,000-30,000	esterified	$3S,3'S$	9

* Crustaceans are believed to have mostly the $3S,3'S$ form, Krill might be the exception.
** depending on tissues, free or esterified astaxanthin may be found

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3. Bioefficacy of algal astaxanthin.

- In **Red Seabream** *(Chrysophrys major)*, pigmentation efficacy of the **esterified form of natural astaxanthin** was reported **superior to synthetic free astaxanthin** [21,18].

- Recent work conducted in Thailand also showed **superior bioefficacy of astaxanthin from *Haematococcus* over the synthetic form**, in larval and post-larval shrimp *(Penaeus monodon)* diets, leading to higher survival [22]. Survival of shrimp zoea fed diets supplemented with 200 ppm algal astaxanthin was found to be 3 times higher than those fed diets supplemented with the same amount of synthetic astaxanthin. In the case of mysis larvae and post-larvae, the algal astaxanthin diets resulted in 20% and 18% improved survival over the synthetic astaxanthin diets.

- In **salmonids**, our trials (Fig. 3, Fig. 4) have shown that properly cell-broken and gently dried algal meal from *Haematococcus*, resulted **in pigmentation and astaxanthin deposition in the flesh, comparable to that obtained from synthetic astaxanthin, when trout were** fed diets supplemented with equivalent levels of these two pigment sources [23]. Portion-size trout *(Oncorynchus mykiss)* fed the algal astaxanthin (Aquaxan HD algal meal) for 90 days, grew to a higher final weight than those fed the synthetic form, suggesting a **superior bioefficacy**, similar to what has been observed with shrimp. An earlier study found that feeding a diet supplemented with astaxanthin from partly-broken *Haematococcus* cells (60% broken cells), resulted in astaxanthin deposition levels in the flesh of trout which were 260% of the level achieved with non-broken cells and 58% of synthetic astaxanthin [24]. This earlier study had concluded that the lower pigmentation efficacy of algal astaxanthin in trout, compared to the synthetic form, could be attributed to two possible causes: insufficient breakage of cell wall and/or lower absorption rate due to the need for fish to hydrolyse the esterified form into free astaxanthin before it can be absorbed and transferred into the blood and organs [24,25]. Assuming the linearity of the pigmentation efficacy and the percentage of broken cells, an extrapolation of those earlier results would have indicated that diets prepared with 100% broken cells would most likely have resulted in astaxanthin deposition very similar to those obtained with the synthetic astaxanthin. Because of the toughness of their cell walls, *Haematococcus* cysts are difficult to rupture, and since the astaxanthin is enclosed inside those cell walls, it is very important to maximise breakage of the cells, without destruction of the astaxanthin. The processing of Aquaxan HD algal meal has been designed to achieve a thorough rupture of the cell walls to ensure the best bio-availability of algal astaxanthin, while minimising losses. Our very good pigmentation efficacy results and earlier work in Japan which found equivalent pigmentation efficacy between synthetic free astaxanthin and esterified natural astaxanthin in coho salmon *(Oncorhynchus kisutch)* [26], concur with studies in red seabream and indicate that **esterified algal astaxanthin and**

synthetic free astaxanthin have similar pigmentation efficacy in salmon or trout.

Pigmentation and growth results with trout fed algal or synthetic astaxanthin

Fig 3
Astaxanthin deposition in muscle of trout fed 10, 25 or 40 ppm astaxanthin

- ■ Synthetic astaxanthin
- ▲ AQUAXAN algae meal
- ▬ ▬ Linear (Synthetic astaxanthin)
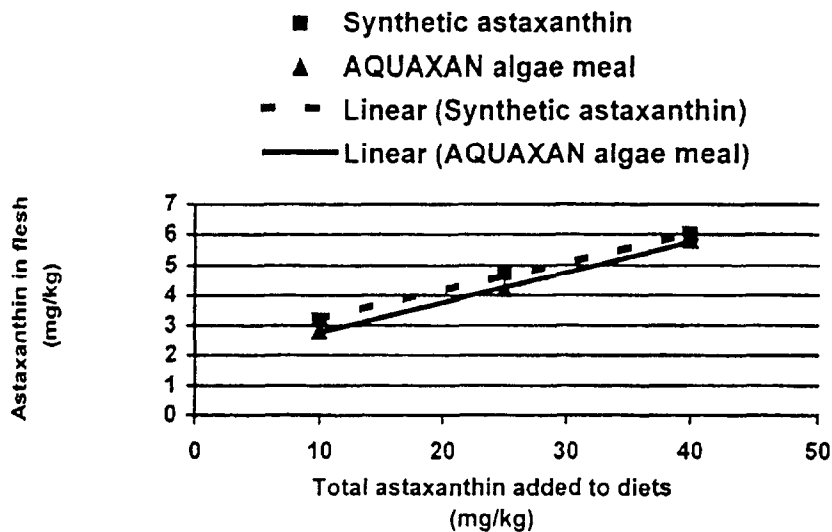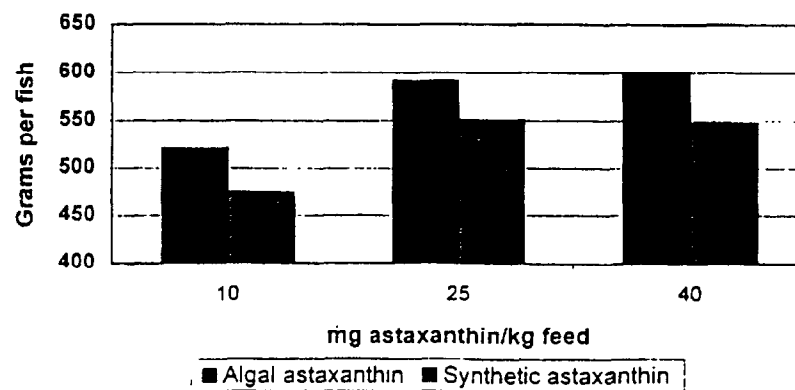- ──── Linear (AQUAXAN algae meal)

Fig.4.

Final weight (g/pc) of fish fed diets supplemented with 10, 25 or 40 ppm algal or synthetic astaxanthin

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4. Functions of astaxanthin:

The main functions of astaxanthin in aquatic species include:
- Pro-vitamin A activity
- Pigmentation, photo response and communication
- Antioxidant properties
- Reproduction and development
- Immune response mechanisms

4.1. Pro-vitamin A activity – role in vision
- Retinoids, including Vitamin A, are well known for their role as vision pigments. In fish, vitamin A has been shown to be an essential vitamin, with deficiency leading to xerophthalmia and cataracts, while supplementation in the diet prevented these deficiency symptoms and promoted growth[27].
- Astaxanthin, as with other carotenoids including beta-carotene, has been reported to play a role as precursor of vitamin A in salmon *(Salmo salar)* and trout *(Oncorhynchus mykiss)* [28,29], tilapia *(Tilapia nilotica)* [30], guppies *(Lebistes reticulatus)* and platies *(Xiphophorus variatus)* [31].
- It should be noted that in the deep sea stomatoid fish *Malacosteus niger*, astaxanthin is a tapetal pigment, believed to function as a diffuse reflector which increases visual sensitivity[32].

4.2. Astaxanthin's role in photoresponse, communication and behaviour
- Fish are known to change coloration in response to changes in lighting and background, during reproductive behaviour or when excited, as both a way of communicating and protecting themselves[33].
- Carotenoid pigments, accumulating and migrating within chromatophores and xanthophores spread out in the tegument of fish, are responsible for these colorations and their changes. It has been suggested that the bright colors of male salmons during reproductive period, resulting from astaxanthin accumulation, is a secondary sexual characteristic that may influence the behaviour and be a condition of the success of the reproductive process[33].
- This role in communication and behaviour is believed to be a major function of carotenoids in the animal world[34].

4.3. Antioxidant properties of astaxanthin: the SUPER VITAMIN E.
- Astaxanthin has been shown to be an excellent natural antioxidant[13,35,36,36,38]. Astaxanthin is very active against singlet oxygen ($^1O_2$), hydroxyl radicals (·OH), and organic free radicals, and was found to be more active than other carotenoids (zeaxanthin, beta-carotene, canthaxanthin) or alpha-tocopherol on those free radical species. Indeed, when compared to vitamin E, the in-vitro activity of astaxanthin was found to be 15 times higher on free radicals, and 100 times on singlet oxygen [13].

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- _In-vitro_ studies have shown astaxanthin to be the most effective natural antioxidant to protect linolenic acid, a polyunsaturated fatty acid (PUFA) from peroxidation[13].   PUFAs are considered critical components of cell membranes of marine fish and shrimp, who have elevated dietary requirements for essential PUFAs.   Those PUFAs are very sensitive to oxidation due to their double bonds.
- Astaxanthin is also believed to protect tissues from photo-oxidation by UV light, e. g , in salmon swimming in shallow waters, or in salmonid eggs.[7]
- Astaxanthin has been attributed a protective effect on some essential vitamins. Feeding trials have shown that tissues of Atlantic salmon (_Salmo salar_) fed astaxanthin-supplemented diets had 2 to 20 times higher levels of physiologically active antioxidant vitamins (retinol, alpha-tocopherol, ascorbic acid) supporting an antioxidant sparing property of astaxanthin on those vitamins[39].

4.4. Role of astaxanthin in reproduction and development.

- Carotenoids, and more specifically astaxanthin, have long been attributed an important role in reproduction of shrimp and fish[7,14].
- It has been noted that astaxanthin deposited in the flesh is mobilised and redeposited in ovaries and the skin during the reproductive cycle of salmonids[14].
- Salmon eggs contain high levels of lipids, specifically polyunsaturated fatty acids (PUFAs), which are critical to the success of reproduction and larval development. It is assumed that astaxanthin plays an important protective role for these PUFAs as a natural _in-situ_ antioxidant[14].
- In red seabream (_Chrysophrys major_), astaxanthin has been found to improve buoyancy and other egg quality parameters- and production of larvae when broodstock were fed diets containing astaxanthin [20]
- Feeding astaxanthin to yellow tail broodstock resulted in improved egg quality [40].
- Recent work has demonstrated that astaxanthin was essential for high survival and rapid growth of newly-hatched salmon fry and juveniles [41,42].

4.5. Effects of astaxanthin on health and immunology

Astaxanthin has been reported to improve both specific and non-specific immune response mechanisms in fish:

- In salmonids, astaxanthin improved survival of Atlantic salmon submitted to an _Aeromonas salmonicida_ challenge, and has been demonstrated to be essential for the survival of salmon fry [43,46].
- _In-vitro_ experiments with trout phagocytes have demonstrated the immuno-stimulatory effect of astaxanthin, which is believed to protect the cell membranes of the phagocytes from oxidation [44].
- Higher astaxanthin levels have been found in phagocytic cells of trout such as phagocytes, macrophages and neutrophils, indicating a greater need for autoprotection against toxic oxidative by-products [41].
- Supplementation of astaxanthin in salmonid diets has been shown to affect _in-vivo_ all non-specific immune response parameters tested [45].
- Those results corroborate a large number of studies which have demonstrated the positive effect of astaxanthin in specific and non-specific immune response

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mechanisms in mammals [46,47,48,49,50,51]. Astaxanthin has been shown to have anticarcinogenic effects in mice[48,51,63,66], to stimulate formation of antibody-forming cells in the spleen of sheep[46,47], to enhance *in-vitro* production of T-cell-dependent antigen in normal strains of mice and possibly antibody production[47].

- Two freshwater fish, *Oreochromis nilotica* and *Colisa labiosa*, fed astaxanthin at 32 or 71 mg/kg, displayed improved histology of the liver, a critical organ which plays an essential role in immune response mechanisms in fish [52]. Astaxanthin supplementation has resulted in improved growth of tilapia [53].
- Finally, the sparing effect of astaxanthin on other essential vitamins with immune response functions may also have an indirect positive effect on the health and immune response of fish[39].

In shrimp, astaxanthin has also been shown to improve survival and immune response.

- In Kuruma shrimp (*P. japonicus*), 50 to 100 ppm dietatry astaxanthin has been shown to improve survival and growth [54,55,57].
- In Tiger prawns (*P. monodon*), 100 to 200 ppm dietary astaxanthin has been shown to improve resistance to bacterial and viral infections[56], while only 50 ppm is sufficient to prevent the blue-shrimp syndrome [16].
- More recently, dietary astaxanthin was shown to improve survival of larval and post-larval shrimp (*P. monodon*), with algal astaxanthin showing a superior effect over the synthetic form[22].

4. Requirements for astaxanthin – recommended supplemental levels

- The increased mortality and reduced growth observed in salmon fed astaxanthin-free diets support the assumption of a vitamin-like property of astaxanthin and of an essential requirement for it[28].
- The minimum requirement for optimal growth and survival of Atlantic salmon fry has been determined to be 5.3 ppm astaxanthin[41]. However to ensure optimal pigmentation, much higher levels are recommended: as high as 50 to 70 mg astaxanthin per kg of feed is common practice in the industry.
- In shrimp, although essentiality of carotenoids seems to be widely accepted [15,58,60], exact requirements have not been determined. Crustaceans, including penaeid shrimp, are able to convert other carotenoids to astaxanthin, although this conversion may be slow or inefficient[58]. Indeed, astaxanthin has been reported to be more effective than beta-carotene and other carotenoids at improving survival[54] and astaxanthin deposition and pigmentation [54,57,58] in peneaid shrimp.
- Dietary levels of 25 to 50 ppm dietary astaxanthin are recommended to correct the blue-shell syndrome of *Penaeus monodon* [16].
- In Kuruma shrimp, *Penaeus japonicus*, it has been found that astaxanthin deposition increased to a maximum 38 mg/kg in the flesh, 85 mg/kg in the head and 54 mg/kg in the shell, when fed up to 200 ppm astaxanthin, with no additional pigmentation efficacy if feeding higher levels than 100 ppm.

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- On the other hand, levels as high as 100 to 400 ppm have been recommended for improved survival, immune response and resistance to disease in shrimp [59,60,61].
- In red seabream, no specific requirements have been determined, but the industry frequently adds up 25 to 50 ppm astaxanthin to commercial diets, since poorly pigmented red seabream have significantly lower marketing acceptance leading to lower selling prices. .
- Astaxanthin is also added to larval and starter diets, often at significantly higher levels than in grower diets. In the case of shrimp larval and postlarval diets, recent work shows that 200 ppm algal astaxanthin is an adequate level and maximises resistance to stress[22].

Table 3   Recommendations on astaxanthin supplemental levels in aquaculture diets.

Suggested supplemental astaxanthin levels (mg/kg feed):				
	Starter/larval diets		Grower diets	
	Low	High	Low	High
Salmonids	30	50	40	80
Red seabream	30	60	30	60
Shrimp	100	200	10	50

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Table 4: Conversion table: algal meal inclusion/supplemental astaxanthin targeted

Supplemental astaxanthin target level (ppm)	Astaxanthin level in Aquaxan HD algae meal				
	1.0%	1.5%	2.0%	2.5%	3.0%
	Inclusion in feed (kg/Ton feed)				
1	0.10	0.07	0.05	0.04	0.03
5	0.50	0.33	0.25	0.20	0.17
10	1.00	0.67	0.50	0.40	0.33
15	1.50	1.00	0.75	0.60	0.50
20	2.00	1.33	1.00	0.80	0.67
30	3.00	2.00	1.50	1.20	1.00
40	4.00	2.67	2.00	1.60	1.33
50	5.00	3.33	2.50	2.00	1.67
60	6.00	4.00	3.00	2.40	2.00
70	7.00	4.67	3.50	2.80	2.33
80	8.00	5.33	4.00	3.20	2.67
90	9.00	6.00	4.50	3.60	3.00
100	10.00	6.67	5.00	4.00	3.33
120	12.00	8.00	6.00	4.80	4.00
140	14.00	9.33	7.00	5.60	4.67
160	16.00	10.67	8.00	6.40	5.33
180	18.00	12.00	9.00	7.20	6.00
200	20.00	13.33	10.00	8.00	6.67
250	25.00	16.67	12.50	10.00	8.33
300	30.00	20.00	15.00	12.00	10.00

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